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By _____

Sir:

Transmitted herewith for filing under 37 CFR §1.53(b) is the
[ ] patent application of
[ ] continuation patent application of
[ ] divisional patent application of
[X] continuation-in-part patent application of

Inventor(s)/Applicant Indentifier: Alessandro SETTE, John SIDNEY and Scott SOUTHWOOD

For: HLA BINDING PEPTIDES AND THEIR USES

[x] This application claims priority from each of the following Application Nos./filing dates:
08/590,298, filed January 23, 1996, the disclosure(s) of which is (are) incorporated by reference.

[ ] Please amend this application by adding the following before the first sentence: --This application is a [ ] continuation [ ] division of and claims the benefit of U.S. Application No. _____, filed _____, the disclosure of which is incorporated by reference.--

Enclosed are:
[X] 2 sheet(s) of [ ] formal [X] informal drawing(s) and 34 pages of specification including description, claims and abstract.
[ ] An assignment of the invention to _____ .
[ ] A [ ] signed [ ] unsigned Declaration & Power of Attorney.
[X] A [ ] signed [X] unsigned Declaration.
[ ] A Power of Attorney by Assignee with Certificate under 37 CFR 3.73(b).
[ ] A verified statement to establish small entity status under 37 CFR 1.9 and 37 CFR 1.27 [ ] is enclosed [ ] was filed in the prior application and small entity status is still proper and desired.
[ ] A certified copy of a _____ application.
[ ] Information Disclosure Statement under 37 CFR 1.97.
[ ] A petition to extend time to respond in the parent application.
[ ] Notification of change of [ ] power of attorney [ ] correspondence address filed in prior application.
[ ] Please cancel claim(s) _____.
[ ]

Pursuant to 37 CFR §1.53(f), Applicant requests deferral of the filing fee until submission of the Missing Parts of Application.

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Respectfully submitted,
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Attorney Docket No. 014137-008050

PATENT APPLICATION

HLA BINDING PEPTIDES AND THEIR USES

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Attorney Docket No. 014137-080500

HLA Binding Peptides and Their Uses

CROSS REFERENCE TO RELATED APPLICATION

This application is continuation in part of U.S. Serial No. 08/590,298, filed January 23, 1996, and is related to U. S. Serial No. 08/753,615, filed November 127, 1996 and U. S. Serial No. 08/452,843, filed May 30, 1995, which is a continuation-in-part of application U.S. Serial No. 08/344,824, filed November 23, 1994, which is a continuation-in-part of application U.S. Serial No. 08/278,634 filed July 21, 1994, all of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

The present invention relates to compositions and methods for preventing, treating or diagnosing a number of pathological states such as viral diseases and cancers. In particular, it provides novel peptides capable of binding selected major histocompatibility complex (MHC) molecules and inducing an immune response.

MHC molecules are classified as either Class I or Class II molecules. Class II MHC molecules are expressed primarily on cells involved in initiating and sustaining immune responses, such as T lymphocytes, B lymphocytes, macrophages, etc. Class II MHC molecules are recognized by helper T lymphocytes and induce proliferation of helper T lymphocytes and amplification of the immune response to the particular immunogenic peptide that is displayed. Class I MHC molecules are expressed on almost all nucleated cells and are recognized by cytotoxic T lymphocytes (CTLs), which then destroy the antigen-bearing cells. CTLs are particularly important in tumor rejection and in fighting viral infections.

The CTL recognizes the antigen in the form of a peptide fragment bound to the MHC class I molecules rather than the intact foreign antigen itself. The antigen must normally be endogenously synthesized by the cell, and a portion of the protein antigen is degraded into small peptide fragments in the cytoplasm. Some of these small peptides

translocate into a pre-Golgi compartment and interact with class I heavy chains to facilitate proper folding and association with the subunit β2 microglobulin. The peptide-MHC class I complex is then routed to the cell surface for expression and potential recognition by specific CTLs.

The MHC class I antigens are encoded by the HLA-A, B, and C loci. HLA-A and HLA-B antigens are expressed at the cell surface at approximately equal densities, whereas the expression of HLA-C is significantly lower (perhaps as much as 10-fold lower). Each of these loci have a number of alleles.

Specific motifs for several of the major HLA-A alleles (copending U.S. Patent Applications 08/159,339 and 08/205,713, referred to here as the copending applications) and HLA-B alleles have been described. Several authors (Melief, *Eur. J. Immunol.*, 21:2963-2970 (1991); Bevan, et al., *Nature* 353:852-955 (1991)) have provided preliminary evidence that class I binding motifs can be applied to the identification of potential immunogenic peptides in animal models. Strategies for identification of peptides or peptide regions capable of interacting with multiple MHC alleles has been described in the literature.

Because human population groups, including racial and ethnic groups, have distinct patterns of distribution of HLA alleles it will be of value to identify motifs that describe peptides capable of binding more than one HLA allele, so as to achieve sufficient coverage of all population groups. The present invention addresses these and other needs.

SUMMARY OF THE INVENTION

The present invention provides compositions comprising immunogenic peptides having binding motifs for HLA alleles. The immunogenic peptides are about 9 to 10 residues in length and comprise conserved residues at certain positions such as a proline at position 2 and an aromatic residue (e.g., Y, W, F) or hydrophobic residue (e.g., L,I,V,M, or A) at the carboxy terminus. In particular, an advantage of the peptides of the invention is their ability to bind to two or more different HLA alleles.

The present invention defines positions within a motif enabling the selection of peptides that will bind efficiently to more than one HLA-A, HLA-B or HLA-C alleles. Epitopes possessing the motif of the immunogenic peptides have been identified on potential target antigens including hepatitis B core and surface antigens (HBVc, HBVs),

hepatitis C antigens, Epstein-Barr virus antigens, human immunodeficiency type-1 virus (HIV1) Lassa virus, p53 CEA, and Her2/neu. Thus, the invention further provides immunogenic peptides comprising sequences of target antigens.

The peptides of the invention are useful in pharmaceutical compositions for both *in vivo* and *ex vivo* therapeutic and diagnostic applications.

Definitions

The term "peptide" is used interchangeably with "oligopeptide" in the present specification to designate a series of residues, typically L-amino acids, connected one to the other typically by peptide bonds between the alpha-amino and carbonyl groups of adjacent amino acids. The oligopeptides of the invention are less than about 15 residues in length and usually consist of between about 8 and about 11 residues, preferably 9 or 10 residues.

An "immunogenic peptide" is a peptide which comprises an allele-specific motif such that the peptide will bind an MHC molecule and induce a CTL response. Immunogenic peptides of the invention are capable of binding to an appropriate HLA molecule and inducing a cytotoxic T cell response against the antigen from which the immunogenic peptide is derived.

A "conserved residue" is a conserved amino acid occupying a particular position in a peptide motif typically one where the MHC structure may provide a contact point with the immunogenic peptide. One to three, typically two, conserved residues within a peptide of defined length defines a motif for an immunogenic peptide. These residues are typically in close contact with the peptide binding groove, with their side chains buried in specific pockets of the groove itself.

The term "motif" refers to the pattern of residues in a peptide of defined length, usually about 8 to about 11 amino acids, which is recognized by a particular MHC allele. The peptide motifs are typically different for each human MHC allele.

The term "supermotif" refers to motifs that, when present in an immunogenic peptide, allow the peptide to bind more than one HLA antigen. The supermotif preferably is recognized by at least one HLA allele having a wide distribution in the human population, preferably recognized by at least two alleles, more preferably

recognized by at least three alleles, and most preferably recognized by more than three alleles.

The phrases "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany it as found in its native state. Thus, the peptides of this invention do not contain materials normally associated with their *in situ* environment, e.g., MHC I molecules on antigen presenting cells. Even where a protein has been isolated to a homogenous or dominant band, there are trace contaminants in the range of 5-10% of native protein which co-purify with the desired protein. Isolated peptides of this invention do not contain such endogenous co-purified protein.

The term "residue" refers to an amino acid or amino acid mimetic incorporated in an oligopeptide by an amide bond or amide bond mimetic.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows binding motifs for peptides capable of binding HLA alleles sharing the B7-like specificity.

Figure 2 shows the B7-like cross-reactive motif.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention relates to the determination of allele-specific peptide motifs for human Class I MHC (sometimes referred to as HLA) allele subtypes. In particular, the invention provides motifs that are common to peptides bound by more than one HLA allele. By a combination of motif identification and MHC-peptide interaction studies, peptides useful for peptide vaccines have been identified.

Following the methods described in the copending applications noted above, certain peptides capable of binding at multiple HLA alleles which possess a common motif have been identified. The motifs of those peptides can be characterized as follows: N-XPXXXXXX(AVILM)-C; N-XPXXXXXXX(AVILM)-C; N-XPXXXXXX(FWY)-C; and N-XPXXXXXXX(FWY)-C. Motifs that are capable of binding at multiple alleles are referred to here as "supermotifs." The particular supermotifs above are specifically called "B7-like-supermotifs."

Immunogenic peptides of the invention are typically identified using a computer to scan the amino acid sequence of a desired antigen for the presence of the supermotifs. Examples of antigens include viral antigens and antigens associated with cancer. An antigen associated with cancer is an antigen, such as a melanoma antigen, that is characteristic of (i.e., expressed by) cells in a malignant tumor but not normally expressed by healthy cells. Examples of suitable antigens particularly include hepatitis B core and surface antigens (HBVc, HBVs) hepatitis C antigens, Epstein-Barr virus antigens, and human immunodeficiency virus (HIV) antigens, and also include prostate specific antigen (PSA), melanoma antigens (e.g., MAGE-1), human papilloma virus (HPV) antigens Lassa virus, p53 CEA, and Her2/neu; this list is not intended to exclude other sources of antigens.

Peptides comprising the supermotif sequences, including those found in proteins from potential antigenic sources are synthesized and then tested for their ability to bind to the appropriate MHC molecules in a variety of assays. The assays may use, for example, purified class I molecules and radioiodonated peptides. Alternatively, binding to cells expressing empty class I molecules can be detected by, for instance, immunofluorescent staining and flow microfluorimetry. Those peptides that bind to the class I molecule may be further evaluated for their ability to serve as targets for CTLs derived from infected or immunized individuals, as well as for their capacity to induce primary *in vitro* or *in vivo* CTL responses that can give rise to CTL populations capable of reacting with virally infected target cells or tumor cells as therapeutic agents.

Recent evidence suggests however, that high affinity MHC binders might be, in most instances, immunogenic, suggesting that peptide epitopes might be selected on the basis of MHC binding alone.

Peptides comprising the supermotif sequences can be identified, as noted above, by screening potential antigenic sources. Useful peptides can also be identified by synthesizing peptides with systematic or random substitution of the variable residues in the supermotif, and testing them according to the assays provided. As demonstrated below, it is useful to refer to the sequences of the target HLA molecule, as well.

The nomenclature used to describe peptide compounds follows the conventional practice wherein the amino group is presented to the left (the N-terminus) and the carboxyl group to the right (the C-terminus) of each amino acid residue. In the

formulae representing selected specific embodiments of the present invention, the amino- and carboxyl-terminal groups, although not specifically shown, are in the form they would assume at physiologic Ph values, unless otherwise specified. In the amino acid structure formulae, each residue is generally represented by standard three letter or single letter designations. The L-form of an amino acid residue is represented by a capital single letter or a capital first letter of a three-letter symbol, and the D-form for those amino acids having D-forms is represented by a lower case single letter or a lower case three letter symbol. Glycine has no asymmetric carbon atom and is simply referred to as "Gly" or G. The letter X in a motif represents any of the 20 amino acids found in Table 1, as well non-naturally occurring amino acids or amino acid mimetics. Brackets surrounding more than one amino acid indicates that the motif includes any one of the amino acids. For example, the supermotif "N-XPXXXXXX(AVILM)-C" includes each of the following peptides: N-XPXXXXXXA-C, N-XPXXXXXXV-C, N-XPXXXXXXI-C, N-XPXXXXXXL-C, and N-XPXXXXXXM-C.

For peptide-based vaccines, the peptides of the present invention preferably comprise a motif which binds a number of HLA alleles which are well-represented in the population. Table 2 shows the distribution of certain HLA alleles in human populations.

TABLE 1

Original Residue	Exemplary Substitution
Ala	Ser
Arg	Lys
Asn	Gln
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Pro
His	Arg; Lys
Ile	Leu; Val; Met
Leu	Ile; Val; Met
Lys	Arg
Met	Leu; Ile; Val
Phe	Tyr; Trp
Ser	Thr
Thr	Ser
Trp	Tyr; Phe
Tyr	Trp; Phe
Val	Ile; Leu; Met
Pro	Gly

TABLE 2

Summary of Population Coverage by Currently Available Assays

			Phenotypic (Allelic) Frequency				
Antigen	HLA Allele	Cell Line(s)	Caucasian	Negro	Japanese	Chinese	Hispanic
A1	A*0101	Steinlin	28.6	10.1	1.4	9.2	10.1
A2.1	A*0201	JY	45.8	30.3	42.4	54.0	43.0
A3.2	A*0301	GM3107	20.6	16.3	1.2	7.1	14.8
A11	A*1101	BVR	9.9	3.8	19.7	33.1	7.3
A24	A*2401	KT3	16.8	8.8	58.1	32.9	26.7
All A			88.9	59.8	91.6	94.6	80.2
B7	B*0701	GM3107	17.7	15.5	9.6	6.9	11.8
B8	B*0801	Steinlin	18.1	6.3	0.0	3.6	9.0
B27	B*2705	LG2	7.5	2.6	0.8	3.4	4.9
B35	B*3503	BHM	15.4	14.8	15.4	9.8	28.1
B54	B*5401	KT3	0.0	0.0	12.4	8.6	0.0
All B			51.9	36.5	35.6	30.2	48.7
Cw6	Cw0601	C1R	17.6	13.7	2.2	19.0	12.2
TOTAL			95.7	76.5	94.7	96.6	91.0

For assays of peptide-HLA interactions (e.g., quantitative binding assays) cells with defined MHC molecules are useful. A large number of cells with defined MHC molecules, particularly MHC Class I molecules, are known and readily available. For example, human EBV-transformed B cell lines have been shown to be excellent sources for the preparative isolation of class I and class II MHC molecules. Well-characterized cell lines are available from private and commercial sources, such as American Type Culture Collection ("Catalogue of Cell Lines and Hybridomas," 6th edition (1988) Rockville, Maryland, U.S.A.); National Institute of General Medical Sciences 1990/1991 Catalog of Cell Lines (NIGMS) Human Genetic Mutant Cell Repository, Camden, NJ; and ASHI Repository, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115. Cell lines suitable as sources for various HLA-A alleles are described in the copending applications. Table 3 lists some B cell lines suitable for use as sources for HLA-B and HLA-C alleles, which are particularly useful in the present invention. All of these cell lines can be grown in large batches and are therefore useful for large scale production of MHC molecules. One of skill will recognize that these are merely exemplary cell lines and that many other cell sources can be employed.

TABLE 3

HUMAN CELL LINES (HLA-B and HLA-C SOURCES)

HLA-B allele	B cell line
B1801	DVCAF
B3503	EHM
B0701	GM3107
B1401	LWAGS
B5101	KAS116
B5301	AMAI
B0801	MAT
B2705	LG2
B5401	KT3
B1302	CBUF
B4403	PITOUT
B3502	TISI
B3501	BUR
B4001	LB

HLA-C allele	B cell line
Cw0601	C1R

In the typical case, immunoprecipitation is used to isolate the desired allele. A number of protocols can be used, depending upon the specificity of the antibodies used. For example, allele-specific mAb reagents can be used for the affinity purification of the HLA-A, HLA-B, and HLA-C molecules. Monoclonal antibodies available for isolating various HLA molecules include those listed in Table 4. Affinity columns prepared with these mAbs using standard techniques are used to purify the respective HLA allele products.

TABLE 4

ANTIBODY REAGENTS

anti-HLA	Name	
HLA-A2	BB7.2	
HLA-A1	12/18	
HLA-A3	GAPA3	(ATCC,HB122)
HLA-11,24.1	A11.1M	(ATCC, HB164)
HLA-A,B,C	W6/32	(ATCC, HB95)
monomorphic	B9.12.1	
HLA-B,C	B.1.23.2	
monomorphic		

The capacity to bind MHC Class I molecules is measured in a variety of different ways. One means is a Class I molecular binding assay as described in Example 2, below. Other alternatives described in the literature include inhibition of antigen presentation (Sette, et al., *J. Immunol.* 141:3893 (1991)), *in vitro* assembly assays (Townsend, et al., *Cell* 62:285 (1990)), and FACS based assays using mutated cells, such as RMA.S (Melief, et al., *Eur. J. Immunol.* 21:2963 (1991)).

Next, peptides that test positive in the MHC class I binding assay are assayed for the ability of the peptides to induce specific CTL responses *in vitro*. For instance, antigen-presenting cells that have been incubated with a peptide can be assayed for the ability to induce CTL responses in responder cell populations. Antigen-presenting cells can be normal cells such as peripheral blood mononuclear cells or dendritic cells (Inaba, et al., *J. Exp. Med.* 166:182 (1987); Boog, *Eur. J. Immunol.* 18:219 (1988)). Alternatively, transgenic mice comprising an appropriate HLA transgene can be used to

assay the ability of a peptide to induce a response in cytotoxic T lymphocytes essentially as described in copending U.S. Patent Application No. 08/205,713.

Alternatively, mutant mammalian cell lines that are deficient in their ability to load class I molecules with internally processed peptides, such as the mouse cell lines RMA-S (Kärre, et al.. *Nature*, 319:675 (1986); Ljunggren, et al., *Eur. J. Immunol.* 21:2963-2970 (1991)), and the human T cell hybridoma, T-2 (Cerundolo, et al., *Nature* 345:449-452 (1990)) and which have been transfected with the appropriate human class I genes are conveniently used, when peptide is added to them, to test for the capacity of the peptide to induce *in vitro* primary CTL responses. Other eukaryotic cell lines which could be used include various insect cell lines such as mosquito larvae (ATCC cell lines CCL 125, 126, 1660, 1591, 6585, 6586), silkworm (ATTC CRL 8851), armyworm (ATCC CRL 1711), moth (ATCC CCL 80) and Drosophila cell lines such as a Schneider cell line (see Schneider *J. Embryol. Exp. Morphol.* 27:353-365 [1927]).

Peripheral blood lymphocytes are conveniently isolated following simple venipuncture or leukapheresis of normal donors or patients and used as the responder cell sources of CTL precursors. In one embodiment, the appropriate antigen-presenting cells are incubated with 10-100 μM of peptide in serum-free media for 4 hours under appropriate culture conditions. The peptide-loaded antigen-presenting cells are then incubated with the responder cell populations *in vitro* for 7 to 10 days under optimized culture conditions. Positive CTL activation can be determined by assaying the cultures for the presence of CTLs that kill radiolabeled target cells, both specific peptide-pulsed targets as well as target cells expressing endogenously processed form of the relevant virus or tumor antigen from which the peptide sequence was derived.

Specificity and MHC restriction of the CTL is determined by testing against different peptide target cells expressing appropriate or inappropriate human MHC class I. The peptides that test positive in the MHC binding assays and give rise to specific CTL responses are referred to herein as immunogenic peptides.

The immunogenic peptides can be prepared synthetically, or by recombinant DNA technology. Although the peptide will preferably be substantially free of other naturally occurring host cell proteins and fragments thereof, in some embodiments the peptides can be synthetically conjugated to native fragments or particles.

The polypeptides or peptides can be a variety of lengths, either in their neutral (uncharged) forms or in forms which are salts, and either free of modifications such as glycosylation, side chain oxidation, or phosphorylation or containing these modifications, subject to the condition that the modification not destroy the biological activity of the polypeptides as herein described.

Desirably, the peptide will be as small as possible while still maintaining substantially all of the biological activity of the large peptide. When possible, it may be desirable to optimize peptides of the invention to a length of 9 or 10 amino acid residues, commensurate in size with endogenously processed viral peptides or tumor cell peptides that are bound to MHC class I molecules on the cell surface.

Peptides having the desired activity may be modified as necessary to provide certain desired attributes, e.g., improved pharmacological characteristics, while increasing or at least retaining substantially all of the biological activity of the unmodified peptide to bind the desired MHC molecule and activate the appropriate T cell. For instance, the peptides may be subject to various changes, such as substitutions, either conservative or non-conservative, where such changes might provide for certain advantages in their use, such as improved MHC binding. By conservative substitutions is meant replacing an amino acid residue with another which is biologically and/or chemically similar, e.g., one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as Gly, Ala; Val, Ile, Leu, Met; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. The effect of single amino acid substitutions may also be probed using D-amino acids. Such modifications may be made using well known peptide synthesis procedures, as described in e.g., Merrifield, *Science* 232:341-347 (1986), Barany and Merrifield, *The Peptides*, Gross and Meienhofer, eds. (N.Y., Academic Press), pp. 1-284 (1979); and Stewart and Young, *Solid Phase Peptide Synthesis*, (Rockford, Ill., Pierce), 2d Ed. (1984), incorporated by reference herein.

The peptides can also be modified by extending or decreasing the compound's amino acid sequence, e.g., by the addition or deletion of amino acids. The peptides or analogs of the invention can also be modified by altering the order or composition of certain residues, it being readily appreciated that certain amino acid residues essential for biological activity, e.g., those at critical contact sites or conserved residues, may generally not be altered without an adverse effect on biological activity.

The non-critical amino acids need not be limited to those naturally occurring in proteins, such as L-α-amino acids, or their D-isomers, but may include non-protein amino acids as well, such as β-γ-δ-amino acids, as well as many derivatives of L-α-amino acids.

Typically, a series of peptides with single amino acid substitutions are employed to determine the effect of electrostatic charge, hydrophobicity, etc. on binding. For instance, a series of positively charged (e.g., Lys or Arg) or negatively charged (e.g., Glu) amino acid substitutions are made along the length of the peptide revealing different patterns of sensitivity towards various MHC molecules and T cell receptors. In addition, multiple substitutions using small, relatively neutral moieties such as Ala, Gly, Pro, or similar residues may be employed. The substitutions may be homo-oligomers or hetero-oligomers. The number and types of residues which are substituted or added depend on the spacing necessary between essential contact points and certain functional attributes which are sought (e.g., hydrophobicity versus hydrophilicity). Increased binding affinity for an MHC molecule or T cell receptor may also be achieved by such substitutions, compared to the affinity of the parent peptide. In any event, such substitutions should employ amino acid residues or other molecular fragments chosen to avoid, for example, steric and charge interference which might disrupt binding.

Amino acid substitutions are typically of single residues. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final peptide. Substitutional variants are those in which at least one residue of a peptide has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with Table 1 when it is desired to finely modulate the characteristics of the peptide.

Substantial changes in function (e.g., affinity for MHC molecules or T cell receptors) are made by selecting substitutions that are less conservative than those in Table 1, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the peptide backbone in the area of the substitution, for example as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in peptide properties will be those in which (a) hydrophilic residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by)

any other residue; (c) a residue having an electropositive side chain, e.g., lysl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g. glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g. phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine.

The peptides may also comprise isosteres of two or more residues in the immunogenic peptide. An isostere as defined here is a sequence of two or more residues that can be substituted for a second sequence because the steric conformation of the first sequence fits a binding site specific for the second sequence. The term specifically includes peptide backbone modifications well known to those skilled in the art. Such modifications include modifications of the amide nitrogen, the α-carbon, amide carbonyl, complete replacement of the amide bond, extensions, deletions or backbone crosslinks. See, generally, Spatola, *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, Vol. VII (Weinstein ed., 1983).

Modifications of peptides with various amino acid mimetics or D-amino acids, for instance at the N- or C- termini, are particularly useful in increasing the stability of the peptide *in vivo*. Stability can be assayed in a number of ways. For instance, peptidases and various biological media, such as human plasma and serum, have been used to test stability. *See, e.g.*, Verhoef et al., *Eur. J. Drug Metab. Pharmacokin.* 11:291-302 (1986). Half life of the peptides of the present invention is conveniently determined using a 25% human serum (v/v) assay. The protocol is generally as follows. Pooled human serum (Type AB, non-heat inactivated) is delipidated by centrifugation before use. The serum is then diluted to 25% with RPMI tissue culture media and used to test peptide stability. At predetermined time intervals a small amount of reaction solution is removed and added to either 6% aqueous trichloracetic acid or ethanol. The cloudy reaction sample is cooled (4°C) for 15 minutes and then spun to pellet the precipitated serum proteins. The presence of the peptides is then determined by reversed-phase HPLC using stability-specific chromatography conditions.

The peptides of the present invention or analogs thereof which have CTL stimulating activity may be modified to provide desired attributes other than improved serum half life. For instance, the ability of the peptides to induce CTL activity can be enhanced by linkage to a sequence which contains at least one epitope that is capable of inducing a T helper cell response. Particularly preferred immunogenic peptides/T helper

conjugates are linked by a spacer molecule. The spacer is typically comprised of relatively small, neutral molecules, such as amino acids or amino acid mimetics, which are substantially uncharged under physiological conditions and may have linear or branched side chains. The spacers are typically selected from, e.g., Ala, Gly, or other neutral spacers of nonpolar amino acids or neutral polar amino acids. It will be understood that the optionally present spacer need not be comprised of the same residues and thus may be a hetero- or homo-oligomer. When present, the spacer will usually be at least one or two residues, more usually three to six residues. Alternatively, the CTL peptide may be linked to the T helper peptide without a spacer.

The immunogenic peptide may be linked to the T helper peptide either directly or via a spacer either at the amino or carboxy terminus of the CTL peptide. The amino terminus of either the immunogenic peptide or the T helper peptide may acylated. Exemplary T helper peptides include tetanus toxoid 830-843, influenza 307-319, malaria circumsporozoite 382-398 and 378-389.

In some embodiments it may be desirable to include in the pharmaceutical compositions of the invention at least one component which primes CTL. Lipids have been identified as agents capable of priming CTL *in vivo* against viral antigens. For example, palmitic acid residues can be attached to the alpha and epsilon amino groups of a Lys residue and then linked, e.g., via one or more linking residues such as Gly, Gly-Gly-, Ser, Ser-Ser, or the like, to an immunogenic peptide. The lipidated peptide can then be injected directly in a micellar form, incorporated into a liposome or emulsified in an adjuvant, e.g., incomplete Freund's adjuvant. In a preferred embodiment a particularly effective immunogen comprises palmitic acid attached to alpha and epsilon amino groups of Lys, which is attached via linkage, e.g., Ser-Ser, to the amino terminus of the immunogenic peptide.

As another example of lipid priming of CTL responses, *E. coli* lipoproteins, such as tripalmitoyl-S-glycerylcysteinlyseryl-serine (P_3CSS) I can be used to prime virus specific CTL when covalently attached to an appropriate peptide. See, Deres et al., *Nature* 342:561-564 (1989), incorporated herein by reference. Peptides of the invention can be coupled to P_3CSS, for example, and the lipopeptide administered to an individual to specifically prime a CTL response to the target antigen. Further, as the induction of neutralizing antibodies can also be primed with P_3CSS conjugated to a peptide which

displays an appropriate epitope, the two compositions can be combined to more effectively elicit both humoral and cell-mediated responses to infection.

In addition, additional amino acids can be added to the termini of a peptide to provide for ease of linking peptides one to another, for coupling to a carrier support, or larger peptide, for modifying the physical or chemical properties of the peptide or oligopeptide, or the like. Amino acids such as tyrosine, cysteine, lysine, glutamic or aspartic acid, or the like, can be introduced at the C- or N-terminus of the peptide or oligopeptide. Modification at the C terminus in some cases may alter binding characteristics of the peptide. In addition, the peptide or oligopeptide sequences can differ from the natural sequence by being modified by terminal-NH_2 acylation, e.g., by alkanoyl (C_1-C_{20}) or thioglycolyl acetylation, terminal-carboxyl amidation, e.g., ammonia, methylamine, etc. In some instances these modifications may provide sites for linking to a support or other molecule.

The peptides of the invention can be prepared in a wide variety of ways. Because of their relatively short size, the peptides can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. See, for example, Stewart and Young, *Solid Phase Peptide Synthesis*, 2d. ed., Pierce Chemical Co. (1984), *supra*.

Alternatively, recombinant DNA technology may be employed wherein a nucleotide sequence which encodes an immunogenic peptide of interest is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression. These procedures are generally known in the art, as described generally in Sambrook et al., *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1982), which is incorporated herein by reference. Thus, fusion proteins which comprise one or more peptide sequences of the invention can be used to present the appropriate T cell epitope.

As the coding sequence for peptides of the length contemplated herein can be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci et al., *J. Am. Chem. Soc.* 103:3185 (1981), modification can be made simply by substituting the appropriate base(s) for those encoding the native peptide sequence. The coding sequence can then be provided with appropriate linkers and ligated into expression

vectors commonly available in the art, and the vectors used to transform suitable hosts to produce the desired fusion protein. A number of such vectors and suitable host systems are now available. For expression of the fusion proteins, the coding sequence will be provided with operably linked start and stop codons, promoter and terminator regions and usually a replication system to provide an expression vector for expression in the desired cellular host. For example, promoter sequences compatible with bacterial hosts are provided in plasmids containing convenient restriction sites for insertion of the desired coding sequence. The resulting expression vectors are transformed into suitable bacterial hosts. Of course, yeast or mammalian cell hosts may also be used, employing suitable vectors and control sequences.

The peptides of the present invention and pharmaceutical and vaccine compositions thereof are useful for administration to mammals, particularly humans, to treat and/or prevent viral infection and cancer. Examples of diseases which can be treated using the immunogenic peptides of the invention include prostate cancer, hepatitis B, hepatitis C, AIDS, renal carcinoma, cervical carcinoma, lymphoma, CMV and condlyloma acuminatum.

For pharmaceutical compositions, the immunogenic peptides of the invention are administered to an individual already suffering from cancer or infected with the virus of interest. Those in the incubation phase or the acute phase of infection can be treated with the immunogenic peptides separately or in conjunction with other treatments, as appropriate. In therapeutic applications, compositions are administered to a patient in an amount sufficient to elicit an effective CTL response to the virus or tumor antigen and to cure or at least partially arrest symptoms and/or complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on, e.g., the peptide composition, the manner of administration, the stage and severity of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing physician, but generally range for the initial immunization (that is for therapeutic or prophylactic administration) from about 1.0 μg to about 5000 μg of peptide for a 70 kg patient, followed by boosting dosages of from about 1.0 μg to about 1000 μg of peptide pursuant to a boosting regimen over weeks to months depending upon the patient's response and condition by measuring specific CTL activity in the patient's blood. It must be kept in mind that the peptides and compositions of the

present invention may generally be employed in serious disease states, that is, life-threatening or potentially life threatening situations. In such cases, in view of the minimization of extraneous substances and the relative nontoxic nature of the peptides, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these peptide compositions.

For therapeutic use, administration should begin at the first sign of viral infection or the detection or surgical removal of tumors or shortly after diagnosis in the case of acute infection. This is followed by boosting doses until at least symptoms are substantially abated and for a period thereafter. In chronic infection, loading doses followed by boosting doses may be required.

Treatment of an infected individual with the compositions of the invention may hasten resolution of the infection in acutely infected individuals. For those individuals susceptible (or predisposed) to developing chronic infection the compositions are particularly useful in methods for preventing the evolution from acute to chronic infection. Where the susceptible individuals are identified prior to or during infection, for instance, as described herein, the composition can be targeted to them, minimizing need for administration to a larger population.

The peptide compositions can also be used for the treatment of chronic infection and to stimulate the immune system to eliminate virus-infected cells in carriers. It is important to provide an amount of immuno-potentiating peptide in a formulation and mode of administration sufficient to effectively stimulate a cytotoxic T cell response. Thus, for treatment of chronic infection, a representative dose is in the range of about 1.0 μg to about 5000 μg, preferably about 5 μg to 1000 μg for a 70 kg patient per dose. Immunizing doses followed by boosting doses at established intervals, e.g., from one to four weeks, may be required, possibly for a prolonged period of time to effectively immunize an individual. In the case of chronic infection, administration should continue until at least clinical symptoms or laboratory tests indicate that the viral infection has been eliminated or substantially abated and for a period thereafter.

The pharmaceutical compositions for therapeutic treatment are intended for parenteral, topical, oral or local administration. Preferably, the pharmaceutical compositions are administered parenterally, e.g., intravenously, subcutaneously, intradermally, or intramuscularly. Thus, the invention provides compositions for

parenteral administration which comprise a solution of the immunogenic peptides dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilized by conventional, well known sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

In some embodiments it may be desirable to include in the pharmaceutical composition at least one component which enhances priming of CTL. Lipids have been identified as agents capable of enhancing priming of CTL *in vivo* against viral antigens. For example, palmitic acid residues can be attached to the alpha and epsilon amino groups of a Lys residue and then linked, e.g., typically via one or more linking residues such as Gly, Gly-Gly-, Ser, Ser-Ser, or the like, to a synthetic peptide which comprises a class I-restricted CTL epitope. The lipidated peptide can be administered in saline or incorporated into a liposome emulsified in an adjuvant, e.g., incomplete Freund's adjuvant. In a preferred embodiment a particularly effective immunogen comprises palmitic acid attached to alpha and epsilon amino groups of Lys, which is attached via linkage, e.g., Ser-Ser, to the amino terminus of a class I restricted peptide having T cell determinants, such as those peptides described herein as well as other peptides which have been identified as having such determinants.

As another example of lipid priming of CTL responses, *E. coli* lipoprotein, such as tripalmitoyl-S-glycerylcysteinly-seryl-serine (P_3CSS), can be used to prime virus specific CTL when covalently attached to an appropriate peptide. See, Deres et al., *Nature* 342:561-564 (1989), incorporated herein by reference. Peptides of the invention can be coupled to P_3CSS, for example, and the lipopeptide administered to an individual to specifically prime a CTL. Further, as the induction of neutralizing antibodies can also be primed with P_3CSS conjugated to a peptide which displays an appropriate epitope, the two

compositions can be combined to more effectively elicit both humoral and cell-mediated responses to viral infection.

The concentration of CTL stimulatory peptides of the invention in the pharmaceutical formulations can vary widely, i.e., from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

The peptides of the invention may also be administered via liposomes, which serve to target the peptides to a particular tissue, such as lymphoid tissue, or targeted selectively to infected cells, as well as increase the half-life of the peptide composition. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. In these preparations the peptide to be delivered is incorporated as part of a liposome, alone or in conjunction with a molecule which binds to, e.g., a receptor prevalent among lymphoid cells, such as monoclonal antibodies which bind to the CD45 antigen, or with other therapeutic or immunogenic compositions. Thus, liposomes filled with a desired peptide of the invention can be directed to the site of lymphoid cells, where the liposomes then deliver the selected therapeutic/immunogenic peptide compositions. Liposomes for use in the invention are formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of, e.g., liposome size, acid lability and stability of the liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka et al., *Ann. Rev. Biophys. Bioeng.* 9:467 (1980), U.S. Patent Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369, incorporated herein by reference.

For targeting to the immune cells, a ligand to be incorporated into the liposome can include, e.g., antibodies or fragments thereof specific for cell surface determinants of the desired immune system cells. A liposome suspension containing a peptide may be administered intravenously, locally, topically, etc. in a dose which varies according to, *inter alia*, the manner of administration, the peptide being delivered, and the stage of the disease being treated.

For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient, that is, one or more peptides of the invention, and more preferably at a concentration of 25%-75%.

For aerosol administration, the immunogenic peptides are preferably supplied in finely divided form along with a surfactant and propellant. Typical percentages of peptides are 0.01%-20% by weight, preferably 1%-10%. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. The surfactant may constitute 0.1%-20% by weight of the composition, preferably 0.25-5%. The balance of the composition is ordinarily propellant. A carrier can also be included, as desired, as with, e.g., lecithin for intranasal delivery.

In another aspect the present invention is directed to vaccines which contain as an active ingredient an immunogenically effective amount of an immunogenic peptide as described herein. The peptide(s) may be introduced into a host, including humans, linked to its own carrier or as a homopolymer or heteropolymer of active peptide units. Such a polymer has the advantage of increased immunological reaction and, where different peptides are used to make up the polymer, the additional ability to induce antibodies and/or CTLs that react with different antigenic determinants of the virus or tumor cells. Useful carriers are well known in the art, and include, e.g., thyroglobulin, albumins such as human serum albumin, tetanus toxoid, polyamino acids such as poly(lysine:glutamic acid), influenza, hepatitis B virus core protein, hepatitis B virus recombinant vaccine and the like. The vaccines can also contain a physiologically tolerable (acceptable) diluent such as water, phosphate buffered saline, or saline, and further typically include an adjuvant. Adjuvants such as incomplete Freund's adjuvant, aluminum phosphate, aluminum hydroxide, or alum are materials well known in the art. And, as mentioned above, CTL responses can be

primed by conjugating peptides of the invention to lipids, such as P_3CSS. Upon immunization with a peptide composition as described herein, via injection, aerosol, oral, transdermal or other route, the immune system of the host responds to the vaccine by producing large amounts of CTLs specific for the desired antigen, and the host becomes at least partially immune to later infection, or resistant to developing chronic infection.

Vaccine compositions containing the peptides of the invention are administered to a patient susceptible to or otherwise at risk of viral infection or cancer to elicit an immune response against the antigen and thus enhance the patient's own immune response capabilities. Such an amount is defined to be an "immunogenically effective dose." In this use, the precise amounts again depend on the patient's state of health and weight, the mode of administration, the nature of the formulation, etc., but generally range from about 1.0 μg to about 5000 μg per 70 kilogram patient, more commonly from about 10 μg to about 500 μg mg per 70 kg of body weight.

In some instances it may be desirable to combine the peptide vaccines of the invention with vaccines which induce neutralizing antibody responses to the virus of interest, particularly to viral envelope antigens.

For therapeutic or immunization purposes, nucleic acids encoding one or more of the peptides of the invention can also be admisitered to the patient. A number of methods are conveniently used to deliver the nucleic acids to the patient. For instance, the nulceic acid can be delivered directly, as "naked DNA". This approach is described, for instance, in Wolff *et. al.*, *Science* 247: 1465-1468 (1990) as well as U.S. Patent Nos. 5,580,859 and 5,589,466. The nucleic acids can also be administered using ballistic delivery as described, for instance, in U.S. Patent No. 5,204,253. Particles comprised solely of DNA can be administered. Alternatively, DNA can be adhered to particles, such as gold particles. The nucleci acids can also be delivered complexed to cationic compounds, such as cationic lipids. Lipid-mediated gene delivery methods are described, for instance, in WO 96/18372; WO 93/24640; Mannino and Gould-Fogerite (1988) *BioTechniques* 6(7): 682-691; Rose U.S. Pat No. 5,279,833; WO 91/06309; and Felgner *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84: 7413-7414. The peptides of the invention can also be expressed by attenuated viral hosts, such as vaccinia or fowlpox. This approach involves the use of vaccinia virus as a vector to express nucleotide sequences that encode the peptides of the invention. Upon introduction into an acutely or chronically infected

host or into a noninfected host, the recombinant vaccinia virus expresses the immunogenic peptide, and thereby elicits a host CTL response. Vaccinia vectors and methods useful in immunization protocols are described in, e.g.,U.S. Patent No. 4,722,848, incorporated herein by reference. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover et al. (Nature 351:456-460 (1991)) which is incorporated herein by reference. A wide variety of other vectors useful for therapeutic administration or immunization of the peptides of the invention, e.g., Salmonella typhi vectors and the like, will be apparent to those skilled in the art from the description herein.

A preferred means of administering nucleic acids encoding the peptides of the invention uses minigene constructs encoding multiple epitopes of the invention. To create a DNA sequence encoding the selected CTL epitopes (minigene) for expression in human cells, the amino acid sequences of the epitopes are reverse translated. A human codon usage table is used to guide the codon choice for each amino acid. These epitope-encoding DNA sequences are directly adjoined, creating a continuous polypeptide sequence. To optimize expression and/or immunogenicity, additional elements can be incorporated into the minigene design. Examples of amino acid sequence that could be reverse translated and included in the minigene sequence include: helper T lymphocyte epitopes, a leader (signal) sequence, and an endoplasmic reticulum retention signal. In addition, MHC presentation of CTL epitopes may be improved by including synthetic (e.g. poly-alanine) or naturally-occurring flanking sequences adjacent to the CTL epitopes.

The minigene sequence is converted to DNA by assembling oligonucleotides that encode the plus and minus strands of the minigene. Overlapping oligonucleotides (30-100 bases long) are synthesized, phosphorylated, purified and annealed under appropriate conditions using well known techniques. he ends of the oligonucleotides are joined using T4 DNA ligase. This synthetic minigene, encoding the CTL epitope polypeptide, can then cloned into a desired expression vector.

Standard regulatory sequences well known to those of skill in the art are included in the vector to ensure expression in the target cells. Several vector elements are required: a promoter with a down-stream cloning site for minigene insertion; a polyadenylation signal for efficient transcription termination; an *E. coli* origin of replication; and an *E. coli* selectable marker (e.g. ampicillin or kanamycin resistance). Numerous promoters can be used for this purpose, *e.g.*, the human cytomegalovirus

(hCMV) promoter. *See*, U.S. Patent Nos. 5,580,859 and 5,589,466 for other suitable promoter sequences.

Additional vector modifications may be desired to optimize minigene expression and immunogenicity. In some cases, introns are required for efficient gene expression, and one or more synthetic or naturally-occurring introns could be incorporated into the transcribed region of the minigene. The inclusion of mRNA stabilization sequences can also be considered for increasing minigene expression. It has recently been proposed that immunostimulatory sequences (ISSs or CpGs) play a role in the immunogenicity of DNA vaccines. These sequences could be included in the vector, outside the minigene coding sequence, if found to enhance immunogenicity.

In some embodiments, a bicistronic expression vector, to allow production of the minigene-encoded epitopes and a second protein included to enhance or decrease immunogenicity can be used. Examples of proteins or polypeptides that could beneficially enhance the immune response if co-expressed include cytokines (e.g., IL2, IL12, GM-CSF), cytokine-inducing molecules (e.g. LeIF) or costimulatory molecules. Helper (HTL) epitopes could be joined to intracellular targeting signals and expressed separately from the CTL epitopes. This would allow direction of the HTL epitopes to a cell compartment different than the CTL epitopes. If required, this could facilitate more efficient entry of HTL epitopes into the MHC class II pathway, thereby improving CTL induction. In contrast to CTL induction, specifically decreasing the immune response by co-expression of immunosuppressive molecules (e.g. TGF-β) may be beneficial in certain diseases.

Once an expression vector is selected, the minigene is cloned into the polylinker region downstream of the promoter. This plasmid is transformed into an appropriate *E. coli* strain, and DNA is prepared using standard techniques. The orientation and DNA sequence of the minigene, as well as all other elements included in the vector, are confirmed using restriction mapping and DNA sequence analysis. Bacterial cells harboring the correct plasmid can be stored as a master cell bank and a working cell bank.

Therapeutic quantities of plasmid DNA are produced by fermentation in *E. coli*, followed by purification. Aliquots from the working cell bank are used to inoculate fermentation medium (such as Terrific Broth), and grown to saturation in shaker flasks or a bioreactor according to well known techniques. Plasmid DNA can be purified using standard bioseparation technologies such as solid phase anion-exchange resins supplied by

Quiagen. If required, supercoiled DNA can be isolated from the open circular and linear forms using gel electrophoresis or other methods.

Purified plasmid DNA can be prepared for injection using a variety of formulations. The simplest of these is reconstitution of lyophilized DNA in sterile phosphate-buffer saline (PBS). A variety of methods have been described, and new techniques may become available. As noted above, nucleic acids are conveniently formulated with cationic lipids. In addition, glycolipids, fusogenic liposomes, peptides and compounds referred to collectively as protective, interactive, non-condensing (PINC) could also be complexed to purified plasmid DNA to influence variables such as stability, intramuscular dispersion, or trafficking to specific organs or cell types.

Target cell sensitization can be used as a functional assay for expression and MHC class I presentation of minigene-encoded CTL epitopes. The plasmid DNA is introduced into a mammalian cell line that is suitable as a target for standard CTL chromium release assays. The transfection method used will be dependent on the final formulation. Electroporation can be used for "naked" DNA, whereas cationic lipids allow direct *in vitro* transfection. A plasmid expressing green fluorescent protein (GFP) can be co-transfected to allow enrichment of transfected cells using fluorescence activated cell sorting (FACS). These cells are then chromium-51 labeled and used as target cells for epitope-specific CTL lines. Cytolysis, detected by 51Cr release, indicates production of MHC presentation of minigene-encoded CTL epitopes.

In vivo immunogenicity is a second approach for functional testing of minigene DNA formulations. Transgenic mice expressing appropriate human MHC molecules are immunized with the DNA product. The dose and route of administration are formulation dependent (e.g. IM for DNA in PBS, IP for lipid-complexed DNA). Twenty-one days after immunization, splenocytes are harvested and restimulated for 1 week in the presence of peptides encoding each epitope being tested. These effector cells (CTLs) are assayed for cytolysis of peptide-loaded, chromium-51 labeled target cells using standard techniques. Lysis of target cells sensitized by MHC loading of peptides corresponding to minigene-encoded epitopes demonstrates DNA vaccine function for *in vivo* induction of CTLs.

Antigenic peptides may be used to elicit CTL *ex vivo*, as well. The resulting CTL, can be used to treat chronic infections (viral or bacterial) or tumors in patients that

do not respond to other conventional forms of therapy, or will not respond to a peptide vaccine approach of therapy. *Ex vivo* CTL responses to a particular pathogen (infectious agent or tumor antigen) are induced by incubating in tissue culture the patient's CTL precursor cells (CTLp) together with a source of antigen-presenting cells (APC) and the appropriate immunogenic peptide. After an appropriate incubation time (typically 1-4 weeks), in which the CTLp are activated and mature and expand into effector CTL, the cells are infused back into the patient, where they will destroy their specific target cell (an infected cell or a tumor cell).

The peptides may also find use as diagnostic reagents. For example, a peptide of the invention may be used to determine the susceptibility of a particular individual to a treatment regimen which employs the peptide or related peptides, and thus may be helpful in modifying an existing treatment protocol or in determining a prognosis for an affected individual. In addition, the peptides may also be used to predict which individuals will be at substantial risk for developing chronic infection.

The following example is offered by way of illustration, not by way of limitation.

Example 1

Identification of immunogenic peptides

Using the B7-like-supermotifs identified in the parent applictions described above, sequences from a number of antigens were analyzed for the presence of the motifs. Tables 5-7 provide the results of these searches.

The above examples are provided to illustrate the invention but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference.

Table 5

Peptide	AA	Sequence	Source
1	8	VPLQLPPL	HIV1 REV73
2	8	APTLWARM	HCV 2869
3	8	IPFYGKAI	HCV 1378
4	8	IPLVGAPL	HCV 137
5	8	KPARLIVF	HCV 2608
6	8	LPGCSFSI	HCV 169
7	8	LPRRGPRL	HCV 37
8	8	LPYIEQGM	HCV 1720
9	9	CPKVSFEPI	HIV1 ENV 285
10	9	IPIHYCAPA	HIV1 ENV 293
11	9	HPVHAGPIA	HIV1 GAG 248
12	10	HPRISSEVHI	HIV1 VIF 48
13	10	LPINALSNSL	HCV
14	11	IPYNPQSQGVV	HIV1 POL 883
15	11	APTLWARMILM	HCV 2869
16	9	MPSLTLACL	Lassa np 179
17	9	VPHVIEEVM	Lassa gp 11
18	10	WPYIASRTSI	Lassa np 317
19	9	FPVTPQVPL	HIV nef 84-92 analog
20	9	FPVRPQFPL	HIV nef 84-92 analog
21	9	IPIPSSWAF	HBV ENV 313
22	9	FPIPSSWAF	HBV ENV 313 analog
23	9	IPITSSWAF	HBV ENV 313 analog
24	9	IPILSSWAF	HBV ENV 313 analog
25	9	FPHCLAFSL	HBV POL 541 analog
26	9	LPGCSFSIF	HCV Core 168
27	9	FPGCSFSIF	HCV Core 168 analog
28	9	LPVCSFSIF	HCV Core 168 analog
29	9	LPGCSFSYF	HCV Core 168 analog

30	9	VPISHLYIL	MAGE2 170
31	9	FPISHLYIL	MAGE2 170 analog
32	9	VPISHLYAL	MAGE2 170 analog
33	9	MPVAGLLII	MAGE3 196 analog
34	9	FPVRMQVPL	HIV nef 84-92 analog
35	9	IPIPMSWAF	HBV ENV 313 analog
36	9	FPHCLAFAL	HBV POL 541 analog
37	9	LPGCMFSIF	HCV Core 168 analog
38	9	VPISMLYIL	MAGE2 170 analog
39	9	FPVRPQVPL	HIV nef 84-92
40	9	FPVTMFFAL	HIV nef 84-92 (a)
41	9	FPVTMFFAM	HIV nef 84-92 (a)
42	9	FPVRMFFAF	HIV nef 84-92 (a)
43	9	FPVRMFFAL	HIV nef 84-92 (a)
44	9	FPVTFFFAL	HIV nef 84-92 (a)
45	9	FPVTMQFAF	HIV nef 84-92 (a)
46	9	FPVTMQFAL	HIV nef 84-92 (a)
47	9	FPVTMFSAF	HIV nef 84-92 (a)
48	9	FPVTMFSAL	HIV nef 84-92 (a)
49	9	FPVRPQVPA	HIV nef 84-92 (a)
50	9	FPVRPQVPV	HIV nef 84-92 (a)
51	9	FPVRPQVPI	HIV nef 84-92 (a)
52	9	FPVRPQVPM	HIV nef 84-92 (a)
53	9	FPVRPQVPF	HIV nef 84-92 (a)
54	9	FPVRPQVPW	HIV nef 84-92 (a)
55	9	FPVRPQVPH	HIV nef 84-92 (a)

The peptides listed in Table 6 were identified as described above and are grouped according to pathogen or antigen from which they were derived.

Table 6

HBV

SEQ ID NO	Sequence	Source
56	IPIPSSWAF	ENV.313
57	HPAAMPHLL	POL.429
58	FPHCLAFSYM	POL.530
59	YPALMPLYA	POL.640
60	LPVCAFSSA	X.58

HCV

SEQ ID NO	Sequence	Source
61	LPGCSFSIF	CORE.169

HIV1

SEQ ID NO	Sequence	Source
62	FPVRPQVPL	NEF.89
63	YPLASLRSLF	GAG.552
64	VPLQLPPL	REV.73

Plasmodium falciparum

SEQ ID NO	Sequence	Source
65	TPYAGEPAPF	SSP2.539

MAGE2/3

SEQ ID NO	Sequence	Source
66	MPKAGLLII	MAGE3.196
67	VPISHLYIL	MAGE2.170
68	LPTTMNYPL	MAGE3.71

Her2/neu

SEQ ID NO	Sequence	Source
69	LPQPPICTI	Her2/neu.941
70	LPTNASLSF	Her2/neu.65
71	MPNQAQMRI	Her2/neu.706

Table 7 provides additional peptides identified using the methods described above.

Peptide	AA	Sequence	Antigen	Protein or Molecule			1st Position	B*0702
1292.01	9	SPRTLNAWI	HIV	GAG	180	0.4200		
1292.02	9	KPCVKLTPI	HIV	ENV	130	0.1100		
1292.03	9	SPAIFQSSI	HIV	POL	335	0.3100		
1292.07	10	LPQGWKGSPI	HIV	POL	328	0.0740		
1292.13	9	HPVHAGPIA	HIV	GAG	248	0.1100		
1292.14	9	HPVHAGPII	HIV	GAG	248	0.4100		
1292.17	9	PPVVHGCPL	HIV	NS5	2317	0.0140		
1292.19	10	KPTLHGPTPI	HIV	NS3	1614	0.2600		
1292.20	10	APTLWARMII	HIV	NS5	2835	0.3900		
1292.22	10	LPRRGPRLGI	HIV	Core	37	0.6700		
1292.23	9	SPGQRVEFI	HIV	NS5	2615	0.0140		
1292.24	9	LPGCSFSII	HIV	Core	169	0.1500		
1292.26	10	SPGALVVGVI	HIV	NS4	1887	0.0220		
1292.27	10	TPLLYRLGAI	HIV	NS3	1621	0.0220		
27.0136	9	APAAPTPAA	p53		76	0.3000		
27.0262	10	APAPAAPTPA	p53		74	0.0190		
27.0264	10	APSWPLSSSV	p53		88	0.0230		
28.0418	9	FPWDILFPA	HDV		194	0.0200		
34.0074	8	IPWQRLLL	CEA		13	0.1100		
34.0075	8	RPGVNLSL	CEA		428	0.0720		
34.0081	8	SPGGLREL	HER2/neu			133	0.0550	
34.0084	8	WPDSLPDL	HER2/neu			415	0.0200	
34.0085	8	IPVAIKVL	HER2/neu			748	0.0120	
34.0086	8	SPYVSRLL	HER2/neu			779	0.0440	
34.0087	8	VPIKWMAL	HER2/neu			884	1.4000	
34.0089	8	SPKANKEI	HER2/neu			760	0.0580	
34.0095	8	RPRFRELV	HER2/neu			966	0.0410	
34.0099	8	SPGKNGVV	HER2/neu			1174	0.0230	
34.0110	8	VPISHLYI	MAGE2		170	0.0170		
34.0111	8	MPKTGLLI	MAGE2		196	0.0190		
34.0117	8	MPKAGLLI	MAGE3		196	0.1300		
34.0121	8	APAPSWPL	p53		86	0.0540		
34.0178	9	GPLPAARPI	HER2/neu			1155	0.0550	
34.0180	9	LPTNASLSI	HER2/neu			65	0.0110	
34.0181	9	SPAFDNLYI	HER2/neu			1214	0.0190	
34.0182	9	SPKANKEII	HER2/neu			760	0.0150	
34.0183	9	SPLTSIISI	HER2/neu			649	0.0640	
34.0184	9	SPREGPLPI	HER2/neu			1151	0.1200	
34.0187	9	GPHISYPPI	MAGE3		296	0.0220		
34.0190	9	RPILTIITI	p53		249	0.0460		
34.0192	9	SPQPKKKPI	p53		315	0.0480		
34.0260	10	GPASPLDSTF	HER2/neu			995	0.0110	
34.0265	10	SPREGPLPAI	HER2/neu			1151	0.0660	
34.0268	10	VPISHLYILI	MAGE2		170	0.0150		
34.0271	10	MPKAGLLIII	MAGE3		196	0.0170		
34.0273	10	APAPAPSWPI	p53		84	0.1300		
34.0361	11	SPLDSTFYRSL	HER2/neu			998	0.0640	
34.0362	11	LPAARPAGATL	HER2/neu			1157	0.0140	
34.0365	11	KPYDGIPAREI	HER2/neu			921	0.0430	
34.0368	11	SPLTSIISAVV	HER2/neu			649	0.0250	
34.0374	11	CPSGVKPDLSY	HER2/neu			600	0.0300	
34.0382	11	GPRALIETSYV	MAGE2		274	0.1300		
34.0387	11	MPKAGLLIIVL	MAGE3		196	0.0280		
34.0389	11	GPRALVETSYV	MAGE3			274	0.1900	
34.0390	11	APRMPEAAPPV	p53			63	0.4500	
34.0397	11	SPALNKMFBQI	p53		127	0.1800		

WHAT IS CLAIMED IS:

1. A composition comprising an immunogenic peptide having an B7-like supermotif, which immunogenic peptide is selected from the group consisting of SEQ ID Nos: 1 through 127.

2. The composition of claim 1, wherein the immunogenic peptide has a sequence from hepatitis B virus and is selected from the group consisting of SEQ ID NO: 56 through SEQ ID NO:60.

3. The composition of claim 1, wherein the immunogenic peptide has a sequence from hepatis C virus and is SEQ ID No:61.

4. The composition of claim 1, wherein the immunogenic peptide has a sequence from human immunodeficiency virus and is selected from the group consisting of SEQ ID No:62 through SEQ ID NO:64.

5. The composition of claim 1, wherein the immunogenic peptide has a sequence from *Plasmodium falciparum* and is SEQ ID No:65.

6. The composition of claim 1, wherein the immunogenic peptide has a sequence from MAGE 2 or MAGE 3and is selected from the group consisting of SEQ ID No: 66 through SEQ ID NO:68.

7. The composition of claim 1, wherein the immunogenic peptide has a sequence from He2/neu and is selected from the group consisting of SEQ ID No:69 through SEQ ID NO:71.

ABSTRACT OF THE DISCLOSURE

The present invention provides peptide compositions capable of binding glycoproteins encoded by HLA, HLA-B, and HLA-C alleles and inducing T cell activation in T cells restricted by the HLA allele. The peptides are useful to elicit an immune response against a desired antigen.

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B7-like cross-reactive motif

	1	2	3	4	5	6	7	8	9
+	YFW		YFW	STC	YFW		YFW	YFW A P	
−	A DE G P		DE	QN		DE G	QN A G	DE	

* Motif is comprised of all residues which are "+" or "-" for two or more alleles.

FIGURE 1

Figure 2

B*0702		Position 2 anchor							C-terminal anchor
preferred	RHK, FWY	P	RHK		RHK	RHK	RHK	P, A	A L I V M F W Y
	1		3	4	5	6	7	8	
deleterious	DE, QN, P		DE, P	DE	DE	G, DE	QN	DE	

B*3501		Position 2 anchor							C-terminal anchor
preferred	LIVM, FWY	P	FWY				FWY		A L I V M F W Y
	1		3	4	5	6	7	8	
deleterious	A, G, P				G	G			

B51		Position 2 anchor							C-terminal anchor
preferred	LIVM, FWY	P	FWY	STC	FWY		G	FWY	A L I V M F W Y
	1		3	4	5	6	7	8	
deleterious	AGPDE, RHK, STC				DE	G	DE, QN	G, DE	

B*5301		Position 2 anchor							C-terminal anchor
preferred	LIVM, FWY	P	FWY	STC	FWY		LIVM, FWY	FWY	A L I V M F W Y
	1		3	4	5	6	7	8	
deleterious	A, GP, QN					G	RHK, QN	DE	

B*5401		Position 2 anchor							C-terminal anchor
preferred	FWY	P	FWY, LIVM		LIVM		A, LIVM	FWY, A, P	A L I V M F W Y
	1		3	4	5	6	7	8	
deleterious	GP, QN, DE		G, DE, STC		DE, RHK	DE	QN, G, DE	DE	

Attorney Docket No. 018623-008050US

DECLARATION

As a below named inventor, I declare that:

My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: **HLA BINDING PEPTIDES AND THEIR USES** the specification of which ___ is attached hereto or _x_ was filed on _February 3, 1998_ as Application No. ___ and was amended on ___ (if applicable).

I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56. I claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign applications(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Country	Application No.	Date of Filing	Priority Claimed Under 35 USC 119
			Yes _ No _
			Yes _ No _

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date

I claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, section 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application No.	Date of Filing	Status
08/590,298	January 23, 1996	_ Patented X Pending _ Abandoned
		_ Patented _ Pending _ Abandoned

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I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 1	Signature of Inventor 2	Signature of Inventor 3
Alessandro Sette	**John Sidney**	**Scott Southwood**
Date	Date	Date

DEC.MRG 7/97

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